The Potential for Combination Treatment Using STAT-C Drugs

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Current Hepatitis Reports 2009, **8:**167–174
Current Medicine Group LLC ISSN 1540-3416

Given the limitations of pegylated interferon (IFN) and ribavirin (RBV) therapy for chronic hepatitis C virus (HCV) infection, new antiviral medication and treatment approaches are sought. Specifically targeted antiviral therapies for hepatitis C (STAT-C) refers to the use of these new inhibitors, either in combination with IFN and RBV or other STAT-C agents, to improve therapy for HCV. Although many classes of inhibitors are being developed, NS3 protease and NS5B polymerase inhibitors are likely to be among the first STAT-C agents approved. The preclinical and clinical characteristics of HCV protease and polymerase inhibitors are reviewed in this article. Strengths and weaknesses of each class in the context of developing future all–STAT-C regimens are explored, with a particular focus on viral resistance and the prospects for eliminating IFN and/or RBV.

Introduction

Therapy for chronic hepatitis C virus (HCV) infection saw incremental improvements over the past decade with the introduction of pegylated interferons (PEG-IFN), weight-based ribavirin (RBV), virologic stopping criteria, and improved management of side effects. Despite these advances, the rate of sustained virologic response (SVR) for genotype 1 HCV following PEG-IFN/RBV therapy is about 45% [1]. Gains from further dose and duration modification of IFN-based therapy are likely minimal and fail to address the large number of patients for whom IFN-based therapy is contraindicated [2]. Additionally, several difficult-to-treat populations (eg, African Americans and HIV-1–coinfected individuals) respond significantly less well to IFN-based therapy [3,4].

The shortcomings of IFN-based therapy and a significant drug discovery effort focusing on HCV resulted in an array of virus-specific inhibitors at various stages of development. Specifically targeted antiviral therapy for hepatitis C (STAT-C) refers to the use of compounds targeting HCV proteins or, in some cases, required cellular cofactors to treat persons with hepatitis C. Although initial approaches focus on the addition of STAT-C agents to PEG-IFN/RBV, the ultimate goal is a potent, well-tolerated regimen of multiple STAT-C drugs that can be administered to an expanded population, including those with contraindications to IFN-based therapy. Before this goal can be realized, several hurdles must be overcome, including the vast genetic diversity of HCV, rapid development of drug resistance, and drug interactions in populations (eg, HIV-1–coinfected persons receiving antiretrovirals). In this article, the feasibility and potential of STAT-C combination therapy are examined; major classes of inhibitors are addressed for their potential in future STAT-C combination therapy; and approaches to testing and implementing these regimens are discussed.

Hepatitis C Virology: Implications for Use of STAT-C Agents

HCV is a positive-sense RNA virus of about 9600 nucleotides encoding a single polyprotein, which is then cleaved by host and virally encoded proteases to form functional viral proteins. HCV replication occurs in the cytoplasm within replication complexes that contain viral and cellular proteins. The viral NS5B RNA–dependent RNA polymerase has no proofreading mechanism, resulting in a swarm of closely related viral genomes (referred to as a quasispecies) within an infected person. As a result of this error-prone replication, HCV shows remarkable genetic diversity on a population level, with about 35% difference in nucleotide sequence between viral genotypes and up to 25% difference in subtypes [5]. This genetic diversity presents a challenge to the development and implementation of STAT-C agents.

Although the error-prone polymerase is a large factor in the development of resistance to STAT-C agents, other characteristics of the HCV life cycle further compound these issues. The amount of virus produced in an infected individual is estimated to be 10^{11} to 10^{12} virions per day; about 10- to 100-fold higher than that seen in HIV-1 [6•]. The more viral turnover that occurs, the higher the likelihood that preexisting resistance mutations will

Table 1. Key concepts for hepatitis C NS3 protease inhibitors

Profound, rapid inhibition of HCV replication
> 3 log by day 3 of therapy for most PIs
Adequate drug exposure is key to maximizing activity and limiting resistance
Exposure prioritized over dosing convenience at this stage of development
Rapid selection and outgrowth of resistant variants occurs during monotherapy
Significant inter- and intragenotype variability exists in the activity and resistance barrier of current clinical lead PIs
Cross-resistance and persistence of resistant mutants may limit sequential treatment options
Mutations R155K and A156T/V confer broad PI cross-resistance
Select resistant mutants can remain significant components of the viral quasispecies for years after exposure

HCV—hepatitis C virus; PI—protease inhibitor.

quickly grow out under selective pressure. The HCV genome encodes a single polyprotein and does not have overlapping reading frames, relaxing the constraints on tolerated mutations compared with other chronic viral infections (eg, hepatitis B virus [HBV] and to a lesser extent HIV-1), which have overlapping reading frames. Finally, the life span of infected hepatocytes is shorter for HCV than HBV, with an estimated half-life of days to several months for HCV and months to years for HBV [6•]. In contrast, productively HIV-1–infected CD4 T cells have a half-life of about 1 day with rapid turnover, creating a large "replication space" for HIV when compared with HCV and HBV. One important difference between HCV and both HBV and HIV-1 is the lack of an integrated (HIV-1 proviral DNA) or latent (HBV cccDNA) cellular form that is capable of archiving resistance mutations and reestablishing active infection after antiviral therapy. This characteristic of HCV replication may increase the chances of successful STAT-C combination therapy; however, the impact of potential sanctuary sites (eg, the central nervous system) and prolonged persistence of relatively fit resistant mutants remains to be determined. In sum, these HCV viral characteristics suggest that resistance mutations will preexist and be selected rapidly during STAT-C antiviral therapy.

Protease Inhibitors

Overview

The HCV NS3 protease is essential for viral replication; by cleaving the single viral polyprotein at several sites, the protease creates functional viral proteins. Early recognition of this role in viral replication and the success of similar approaches in HIV-1 therapy led to an intense drug-discovery effort targeted at this protein [7]. However, structural characteristics of the protein proved challenging when specific and potent NS3 protease inhibitors (PI) were sought. The protease active site is shallow, relatively featureless, and exposed—characteristics that make the rational design of inhibitors difficult. Early successes focused on modeling candidate inhibitors after the authentic viral target and inhibitory cleavage products [8]. Following these design paradigms, two structural classes of NS3 PIs emerged: macrocyclic compounds and peptidomimetic ketoamides. The first proof-of-concept study in humans was conducted with BILN-2061 (a macrocyclic), and demonstrated the rapid and profound impact of PIs on HCV viral load [9]. Subsequent human trials confirmed these findings for macrocyclic and ketoamide PIs and provided additional insights. The major concepts regarding PI-based inhibition of HCV replication are discussed below and summarized in Table 1.

Illustrative compounds

Several HCV PIs representing different chemical classes are at various stages in clinical trials. This review focuses on the compounds furthest along in clinical development and on those at earlier stages that appear to have unique and potentially advantageous characteristics pertaining to use in future STAT-C regimens. Two ketoamide HCV PIs—telaprevir and boceprevir—have commenced phase 3 trials after encouraging results in phase 2 trials in combination with PEG-IFN/RBV. The recently published Protease Inhibition for Viral Evaluation (PROVE)-1 and PROVE-2 studies (ClinicalTrials.gov numbers NCT00372385 and NCT00336479, respectively) demonstrated significant increases in SVR rates with 12 weeks of telaprevir, 750 mg three times daily, in combination with 24 weeks of PEG-IFN/RBV, compared with controls treated with PEG-IFN/RBV for 48 weeks (61% vs 41%, $P = 0.02$ and 69% vs 46%, $P = 0.004$ respectively) [10••,11]. Discontinuations because of adverse events, particularly rash, were higher in subjects treated with telaprevir compared with controls in the PROVE-1 (21% vs 11%) and PROVE-2 studies (12% vs 7%). The Serine Protease Inhibitor Therapy-1 (SPRINT-1) study (ClinicalTrials.gov number NCT00705432) assessed boceprevir, 800 mg three times daily, in combination with PEG-IFN/RBV for 28 or 48 weeks (boceprevir was administered for the entire time); SVR rates were 54% and 67% for the durations of 28 and 48 weeks (38% control, $P = 0.013$ and $P < 0.0001$, respectively) and 75% with a 4-week lead-in phase with PEG-IFN/RBV followed by 44 weeks of PEG-IFN/RBV/boceprevir [12]. Treatment discontinuations because of

adverse events were higher in the boceprevir arms (11% in the 24-week arm and 19% in the 48-week arm) compared with controls (8%).

Differences in trial design and patient populations studied preclude direct comparison of SVR rates between the trials; however, it appears that telaprevir and boceprevir comparably and significantly increase SVR rates in naïve HCV genotype 1–infected persons, albeit with increased rates of adverse events. Differences between the compounds focus on adverse effects and potential drug interactions because of different metabolic pathways. Tolerability in clinical practice may be an issue for both compounds, especially when coadministered with PEG-IFN/RBV.

The macrocyclic PI BILN-2061 validated the NS3 protease as an antiviral target, and several macrocyclic PIs are currently in clinical trials [13–15]. In early monotherapy and IFN combination studies, these inhibitors were potent and well tolerated, with the potential for once-daily dosing [15]. Finally, compared with other macrocyclic inhibitors, EA-063, a recently described PI, has smaller increases in median effective concentration (EC_{50}) with several common PI mutations (R155K and D168V) in vitro. The significance of this finding in vivo—particularly in patients who failed other HCV PI therapy—is unknown, given that these mutations still result in a 10- to 500-fold increase in EC_{50} in vitro to EA-063 [16]. These characteristics may offer a higher barrier to resistance rather than activity against established mutants.

Resistance to protease inhibitors

Resistance to HCV NS3 PIs is rapidly selected both in vitro and in patients during monotherapy—or, importantly, in combination with IFN when RBV is omitted or is used at low doses [10••,12,17,18,19•]. Substitution of the alanine at position 156 with serine (A156S) and the aspartic acid at position 168 with valine (D168V) initially were described in vitro to confer resistance to ketoamide and macrocyclic inhibitors, respectively [17]. It was further shown that substitutions of valine or threonine at position 156 (A156T/V) conferred cross-resistance to ketoamide and macrocyclic inhibitors [18]. In vivo, the major HCV PI resistance mutations identified during telaprevir therapy were at serine 156 (A156S>A156T/V), arginine 155 (R155K/T), threonine 54 (T54A), and valine 36 (V36M/A) [19•]. The R155K mutation has emerged frequently in published trials of PI therapy. In vitro, this mutation confers low-level resistance (~ 10×) to ketoamide inhibitors and high-level resistance to macrocyclic inhibitors (> 100×) while maintaining replicative fitness [20]. Based on in vitro and in vivo studies, the R155K mutation confers cross-resistance to all PIs currently in clinical trials and is fit enough that it occasionally was found as a majority species in chronically infected persons before PI exposure [21,22•]. In the PROVE-1 and 2 studies, the R155K mutation (either alone or in combination with V36M) was the most frequent mutation seen in genotype 1a patients with viral breakthrough or relapse after therapy; in genotype 1b, mutations at position 156 tend to be more common because changes at two nucleotides are required for the R155K mutation [10••,11]. Mutations at position 36 confer low-level resistance in isolation; however, the significance of this mutation is that, when combined with mutations at either the 155 or 156 position, high-level resistance is observed with improved replication fitness [19•]. Long-term follow-up data are still being collected, but the R155K, T54A, and V36M mutations persisted for many months to years after the cessation of PI therapy in humans [19•,23].

Pretreatment with PEG-IFN/RBV for several weeks appears to decrease the rate of breakthrough and resistance development to PIs in early trials [12]. With lower replication levels, the ability of the virus to generate mutants is decreased and the outgrowth of preexisting mutants is hampered. PEG-IFN/RBV lead-in therapy may preemptively "pick off" preexisting resistant mutants that are less fit for replication in vivo. Poor or null responders to IFN-based therapy would not be expected to benefit from this approach. This approach is possible with PEG-IFN/RBV because of a lack of treatment-specific resistance mutations. In the future, trials composed solely of direct-acting STAT-C agents will require simultaneous combination therapy with inhibitors of several targets, and/or unique resistance mutations, to present a high combined-resistance barrier, as is the norm for tuberculosis or HIV-1 therapy.

Potential role of protease inhibitors in STAT-C regimens

Given that PIs are farthest along in development, combined with their potency in vivo, they likely will be a prominent component of future STAT-C regimens, particularly for patients infected with genotype-1 HCV. Additionally, high levels (> 20% of the viral quasispecies) of preexisting resistant mutants appear to be relatively rare (1%–5% of individuals with genotype 1) [21,22•]. Viral resistance may be an issue for patients failing an initial PI-based therapy because of cross-resistance conferred by mutations at positions 155 and 156 and prolonged survival of resistant mutants after exposure.

Polymerase Inhibitors

Overview

The HCV NS5B RNA–dependent RNA polymerase is essential for HCV replication, generating copies of both the minus and plus strands of viral RNA during replication. Crystal structures of the polymerase revealed a classic, right-hand configuration RNA polymerase with a highly conserved active site and nucleotide triphosphate binding tunnel [24]. Additional features of the polymerase with implications for STAT-C therapy include the lack of a proofreading mechanism (resulting in a high error rate), conformation change in the transition from initiation to a processive form, requirement of cellular cofactors for efficient replication (eg, cyclophilins), and the existence of multiple allosteric inhibitor binding sites distinct from the

Table 2. Key concepts for hepatitis C NS5B polymerase inhibitors

Nucleoside inhibitors	Nonnucleoside inhibitors
Potent activity with recent nucleosides	Modest activity, multiple targets
2–3 log RNA reduction with monotherapy	1–2 log RNA reduction with monotherapy
Broad cross-genotype activity	Limited cross-genotype activity
Highly conserved polymerase active site	Variability in binding sites
High barrier to resistance	Low barrier to resistance
Mutants with low replicative fitness in vitro	Mutants relatively fit in vitro
Resistance not yet demonstrated in vivo	Rapid resistance in vivo
Preexisting resistance not noted	Preexisting resistance prevalent
Potential interaction with ribavirin if coadministered	Inhibitors from many chemical classes

polymerase active site [6•,25,26]. Because of the existence of the polymerase active site and multiple allosteric sites, two major classes of HCV inhibitors are directed at the NS5B polymerase: nucleoside polymerase inhibitors (NIs) and nonnucleoside polymerase inhibitors (NNIs). General characteristics of polymerase inhibitors as STAT-C agents are detailed below and summarized in Table 2.

Nucleoside polymerase inhibitors

NIs target the NS5B polymerase active site by competitively inhibiting the incorporation of endogenous nucleotide triphosphates in the elongating RNA chain. They function as chain terminators once incorporated into the RNA molecule; however, because most compounds in this class possess a 3′OH, they are termed nonobligate chain terminators [27]. NIs are attractive HCV inhibitors for several reasons, including the highly conserved nature of the polymerase active site, the proven utility of this class of inhibitors in other viral disease, and the high barrier to resistance described in vitro and in early clinical trials [24,28].

Nonnucleoside polymerase inhibitors

NNIs of the HCV polymerase bind to sites unique from the polymerase active site and inhibit its function in a noncompetitive manner with nucleotide triphosphates. At least four NNI binding sites with multiple chemical classes of compounds have been described to inhibit the purified polymerase and HCV replication in vitro [26]. Although specific mechanisms of action are not defined for all NNIs, many appear to exert their effect by inhibiting the conformational change necessary for the polymerase to go from initiation to elongation [29,30].

Illustrative compounds

The first HCV nucleoside inhibitors described in vitro and tested in humans were the 2′-methyl nucleosides, with the prototypical compound being a prodrug of 2′C-methylcytidine [27,31]. Although initial clinical studies clearly demonstrated inhibition of HCV replication, early compounds were hampered by lack of potency and poor tolerability [31]. Second-generation NIs (eg, the 4′azido compounds) had improved potency in vivo (2–3 log decreases in HCV RNA during monotherapy), but were still plagued by poor tolerability, which limited exposure and increased relapse rates [32]. Recently, 2′-deoxy HCV nucleosides were described, and a related compound R7128 (a prodrug of 2′-deoxy-2′-fluoro-2′-C-methylcytidine) initiated phase 2b trials in combination with PEG-IFN/RBV [33]. During a phase 1b study of R7128, 1500 mg orally, twice daily, in combination with PEG-IFN/RBV for 4 weeks, 85% of subjects had an undetectable HCV viral load by the end of administration [34].

Several HCV NNIs were examined in human trials with limited success because of lack of efficacy and tolerability. Agents currently in clinical trials include ANA-598 (a palm site inhibitor) and VCH-916 (a thiophene 2-carboxylic acid derivative thumb site inhibitor) [35,36].

Resistance to polymerase inhibitors

Resistance development to NI and NNI polymerase inhibitors is quite different, with NI resistance difficult to select in vitro and not yet seen in clinical trials. In contrast, NNI resistance is readily selected for both in vitro and in vivo. In vitro, the S282T mutation confers modest resistance (two- to threefold) to 2′-methyl substitutes nucleosides while significantly hampering the replicative fitness of replicons (5%–10% of wild-type) [28]. The S96T ± N142T mutations confer resistance to the NI 4′-azidocytidine in vitro, again with modest changes in EC_{50} and a dramatic loss in replicative fitness (5%) [28]. These features imply why these sites are highly conserved across all HCV genotypes and why mutants do not preexist in HCV-infected patients to any significant degree [37•]. Nucleoside polymerase inhibitors (eg, the recently described 2′-deoxy-4′-azido nucleoside analogs) are active in vitro against the S282T and N96T mutants, with resistance mutations specific to this compound not reported [33].

Resistance to NNIs at all binding sites was described, and the allosteric sites can be identified by their signature resistance mutations [26]. Resistance to NNIs was reviewed [38]. Important features for their utility in STAT-C regimens include a low genetic barrier to resistance, the ability of certain mutations to confer cross-resistance to other allosteric sites (eg, C316Y confers resistance

to inhibitors of both palm domains), and preexisting resistant mutants within the viral quasispecies in a high percentage of naïve patients [37•]. Furthermore, resistant variants retain much of their replication fitness in many instances, and dual resistance to a combination of a palm and thumb inhibitor can be readily selected in vitro [39].

Potential role of polymerase inhibitors in STAT-C regimens

NIs are particularly attractive as a component of an all–STAT-C regimen because of their high resistance barrier, broad genotype activity, and the low fitness/lack of preexistence of polymerase active site mutants. Although early trials validated the target, a lack of potency and side effects precluded the use of these early agents in STAT-C therapy. Current NIs are more potent; however, tolerability over 12 to 24 weeks of administration is unproven. One complicating factor for NIs, should RBV remain an essential component of HCV therapy, is the potential for intracellular phosphorylation interactions between RBV and nucleoside analogs [40].

The large number of allosteric binding sites and the diversity of chemical classes from which NNI inhibitors can be found increase the likelihood that viable anti-HCV drugs may emerge. However, issues such as limited and highly variable activity across and within genotypes may significantly limit the number of patients for which any given NNI may be useful. Pretherapy resistance testing (sequencing) may be necessary to successfully implement NNI therapy in a given patient, a scenario that is likely unnecessary for NIs or PIs.

STAT-C Agents Directed at Other Viral Targets

Inhibitors of several other HCV proteins including NS4A, the NS3 helicase, the HCV internal ribosome entry site, and NS5A are in various stages of development. Although they are not discussed here, the existence of multiple targets with multiple inhibitors increases the likelihood of realizing potent all–STAT-C regimens in the future. Targeting cellular proteins essential for HCV replication offers another means of inhibiting HCV replication. Current leading examples of such inhibitors include nitazoxanide and cyclophilin inhibitors. Nitazoxanide acts through increasing phosphorylation of double-stranded RNA–dependent protein kinase (PKR), ultimately inhibiting viral translation; nitazoxanide inhibits HCV replication in vitro and appears to augment the IFN response when coadministered [41]. Similar to IFN, no resistance mutations were found in HCV replicons during prolonged exposure. Preliminary results were reported in genotype 1 patients treated with combination therapy including nitazoxanide [42]. Nitazoxanide's clinical safety record (administered as an antiprotozoal for prolonged periods in humans) combined with a lack of described resistance make it a potential addition to STAT-C combination therapy, especially if it has an immune-modulating effect in vivo [41].

Cyclophilins are abundant host cellular proteins that interact with the HCV RNA polymerase and are necessary for efficient HCV replication [25]. Debio 025 is a nonimmunosuppressive cyclophilin inhibitor that Is a potent HCV inhibitor in vitro and in vivo (-4.61 log for Debio 025, 600 mg, combined with PEG-IFN vs -2.49 log for PEG-IFN alone for 4 weeks in genotypes 1 and 4) [43]. Resistance to cyclosporine and Debio 025 can be selected in vitro; however, mutations are scattered throughout NS5B and NS5A, and result in only a modest increase in EC_{50} [44]. No resistance mutations were documented in vitro during monotherapy or coadministration with IFN; thus, it appears the resistance barrier for cyclophilin inhibitors is high, similar to that described for NIs. Although these agents do not specifically target HCV viral proteins, they possess properties making them attractive as potential components of a STAT-C combination regimen.

Will an All–STAT-C Regimen Work?

Unique aspects of IFN and RBV

The mechanism by which IFN-based therapy results in cure of chronic HCV is presumably by stimulating cellular antiviral mechanisms and immunomodulation. IFN therapy increases HCV-specific CD4 and CD8 T-cell responses, and these responses are associated with successful HCV therapy [45,46]. Although it seems clear that STAT-C agents can replace and even improve on the antiviral activity of IFN, whether the immunomodulatory effects are critical in achieving SVRs is unknown. HCV itself is immunosuppressive, both by direct interference with key cellular immune-response pathways (eg, NS3 protease cleavage of IFN-β promoter stimulator-1) [47] and through a more nonspecific "immune exhaustion" associated with upregulation of the inhibitory molecule programmed death-1 [48]. Use of STAT-C agents should reverse these two forms of immunosuppression, but whether direct immune stimulation, as occurs with IFN, is needed for a durable cure remains the unanswered question while all–STAT-C regimens are pursued.

RBV also was a key component in preventing resistance and/or breakthrough during STAT-C combination therapy with IFN. Trials with STAT-C agents and PEG-IFN showed dramatic increases in viral breakthrough on therapy (PIs and NIs), significant increases in viral resistance to PIs, and increased relapse rates (PIs and NIs) when RBV is omitted or used at low doses [10••,12,32]. RBV may act as both a mutagen and an immunomodulator and augments the second-phase of viral clearance, which is believed to correlate with infected hepatocyte loss [49]. Although it is now clear that RBV is necessary if a single STAT-C agent is combined with PEG-IFN, additional studies are needed to determine if an additional STAT-C agent can replace RBV.

Future directions

The much publicized INFORM-1 trial (ClinicalTrials.gov number NCT00801255) was the first to assess

combination therapy with two STAT-C inhibitors in HCV-infected humans [50]. The NI R7128 (PSI-6130) was combined with the PI R7227 (ITMN-191) for 14 days. A decrease of about 5 log in HCV RNA was seen in the three highest-dose groups. No viral rebound was seen during therapy, in contrast to PI monotherapy, where viral rebound can occur as early as day 3. This suggests that the addition of a NI with a high barrier to resistance can prevent the emergence of PI-resistant HCV in the short term. One caveat to this assertion is that at this time, details on clonal analysis of viral populations for resistance mutations have not been presented. It also remains to be seen if subjects with breakthrough or relapse in the PEG-IFN/RBV rollover portion of the study will have PI or (less likely) NI resistance mutations in their viral quasispecies. Based on estimates by Perelson [51] from modeling of HCV replication and mutation rates in chronically infected humans, it seems unlikely that two STAT-C agents will be able to present the virus with a high enough resistance barrier to attain prolonged viral suppression and sustained responses.

Taking cues from the INFORM-1 study, what are some of the pressing issues as clinical trials of STAT-C combinations more forward? Developing a framework whereby novel combinations of STAT-C agents can be tested effectively in a timely manner is of utmost importance. Concepts learned during the testing and evolution of HIV-1 antiretroviral therapy must not be forgotten. National and international clinical trials networks, in close collaboration with the pharmaceutical industry and drug regulatory boards, will be necessary to push the field forward while maintaining patient safety. Because drug resistance is likely to be one of the major complications for an all–STAT-C regimen, the prospective, detailed evaluations of resistant viral variants are crucial. However, before theses studies can be accomplished, definitions and testing methodologies for the detection of resistant mutants need to be standardized. The HCV Drug Resistance Advisory Group was established to meet this goal.

Conclusions

Curing HCV infection with a STAT-C regimen presents formidable challenges because of viral characteristics that promote rapid resistance development. However, the large number of diverse candidate viral inhibitors and successful proof-of-concept clinical studies suggest that such a regimen is attainable. Unanswered questions surround the need for IFN and/or RBV as adjuncts to STAT-C agents, and the number of agents required to prevent resistance and viral breakthrough. Although nuances exist in the development and implementation of STAT-C combination therapy, many concepts fundamental to treating chronic viral infections such as HIV-1 undoubtedly apply.

Disclosure

No potential conflict of interest relevant to this article was reported.

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